

A TRANSLATIONAL STUDY OF THE CORRELATION BETWEEN LOW BIRTH
WEIGHT, HYPERTENSION, AND KIDNEY FUNCTION USING A RAT MODEL

by

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ABSTRACT

We studied the correlation between low birth weight, hypertension, and kidney function using a rat model. There is a strong correlation between these three phenomena especially in the Southeastern United States and in non-White populations. We hypothesized that the anti-hypertensive drugs Reserpine and Hydralazine would prevent hypertension and improve renal function in low birth weight rats. We used a rat model created by Dr. Barbara Alexander in this study. Pregnant rats were subjected to Reduced Uterine Perfusion Pressure surgery. Silver clips were placed on the abdominal aorta and uterine arteries approximately two weeks after fertilization in order to restrict blood flow to the developing fetus. The reduced nutrient availability results in slower fetal development and low birth weight offspring. Two drugs with effects on cardiovascular function were used to lower blood pressure. Hydralazine was administered via drinking water at a dose of 80mg/L initiated at 6 weeks of age until the end experiment at 12 weeks of age. Reserpine was administered via drinking water at a dose of 5mg/L initiated at 6 weeks of age until the end of experiments at 12 weeks of age. Administration of this medication constituted the “treated” rats. At 12 weeks of age, catheters were inserted to measure blood pressure and glomerular filtration rate was calculated. Mild ischemia reperfusion was performed to see how the kidneys reacted to mild stress. We found that the uterine restricted rats had normal gestations, but weighed significantly less than the controls at birth. The rats gained weight at the same rate and weighed the same at the end of the study. As expected, low birth weight untreated offspring had higher blood pressure than any other group. Surprisingly, GFR/g in the uterine-restricted, unstressed, untreated animals was not significantly higher, as predicted from both the higher MAP and presumably lower nephron number in these rats. Thus, there was no evidence of significant hyperfiltration occurring, and so this seemingly cannot explain the hypertension which developed. Based on this study, I would advocate using low birth weight as a biomarker for elevated risk of hypertension and kidney disease.

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Introduction

Fetal programming can significantly influence the physiology of adults. Maternal factors such as pre-eclampsia, malnutrition, smoking, alcohol consumption, certain drugs, and disease can affect the offspring's birth weight and health (1). These maternal factors can affect the epigenetics (changes in gene transcription due to environment) of the offspring, which can lead to medical problems over time. Low birth weight (LBW) is an important epigenetic phenomenon that commonly results from a poor fetal environment and has been linked with chronic detrimental effects in adults (Fig 1).

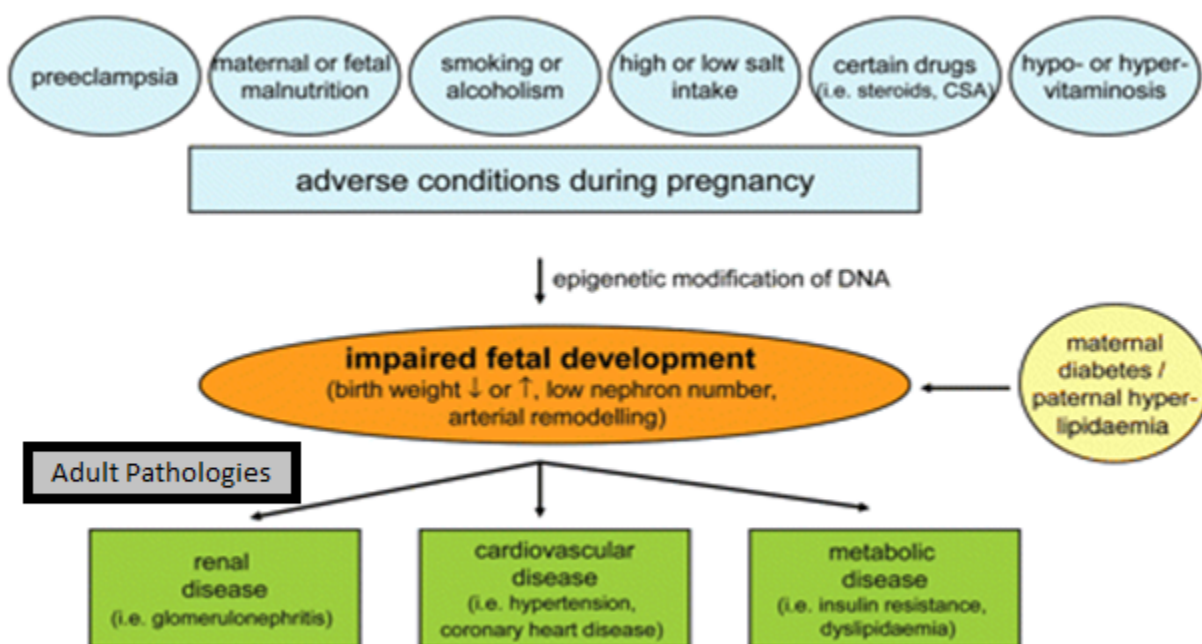
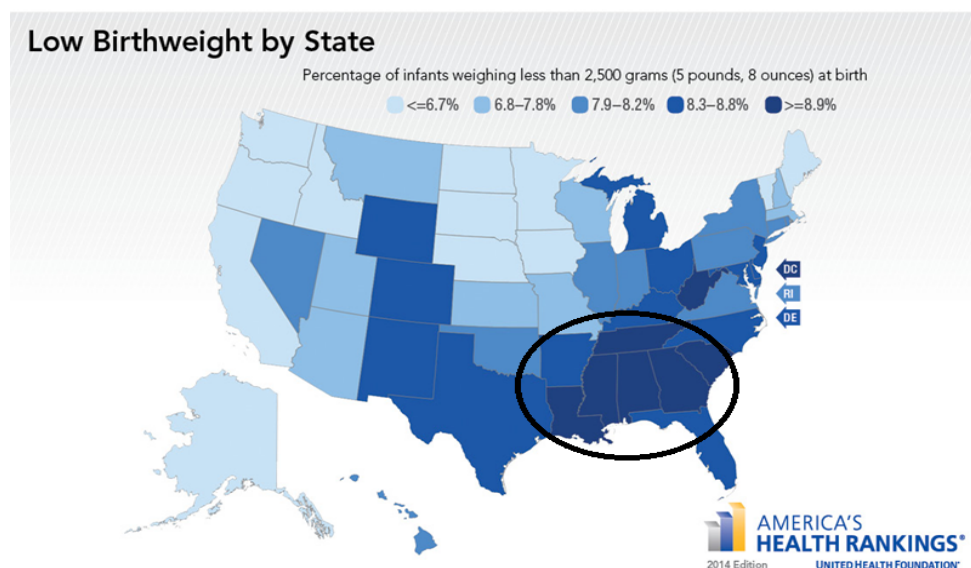


Figure 1. Summary of maternal conditions that may influence fetal development and risk for developing pathophysiological conditions.

This is from N. Koleganova¹, K. Benz, G. Piecha¹, E. Ritz and K. Amann.
From Renal, cardiovascular and metabolic effects of fetal programming
Nephrol Dial Transplant (2012) 27: 3003–3007

For humans, LBW is defined as being born under 5 pounds, 8 ounces. About 1 in every 12 births in the United States meets this criterion (2). Many LBW offspring develop hypertension as adults which can lead to chronic kidney disease (CKD). Hypertension is defined as having systolic pressure at or above 140 mmHg and diastolic pressure at or above 90 mmHg (3). Chronic kidney disease is present when a patient's glomerular filtration rate (GFR) remains below 60 milliliters per minute for more than 3 months or when a patient's urine albumin-to-creatinine ratio is over 30 milligrams of albumin for each gram of creatinine (30 mg/g) (4). It is a condition that damages the kidneys in a circular mechanism. Development of CKD can initiate a pathological positive feedback loop that further contributes to hypertension which further promotes CKD. End stage renal disease (ESRD) is total loss of kidney function. This disease occurs as a result of CKD. Research shows that these phenomena are related.

Understanding the correlation between LBW, hypertension, and CKD is very important for a number of reasons. Estimates show that one in nine Americans, around 20 million, suffer from CKD but are unaware of the condition (5). These individuals can easily develop ESRD which costs \$76,515 per patient per year. There is a strong correlation between regional incidences of LBW, hypertension, and CKD, suggesting a possible causal relationship between these factors (Fig 2).



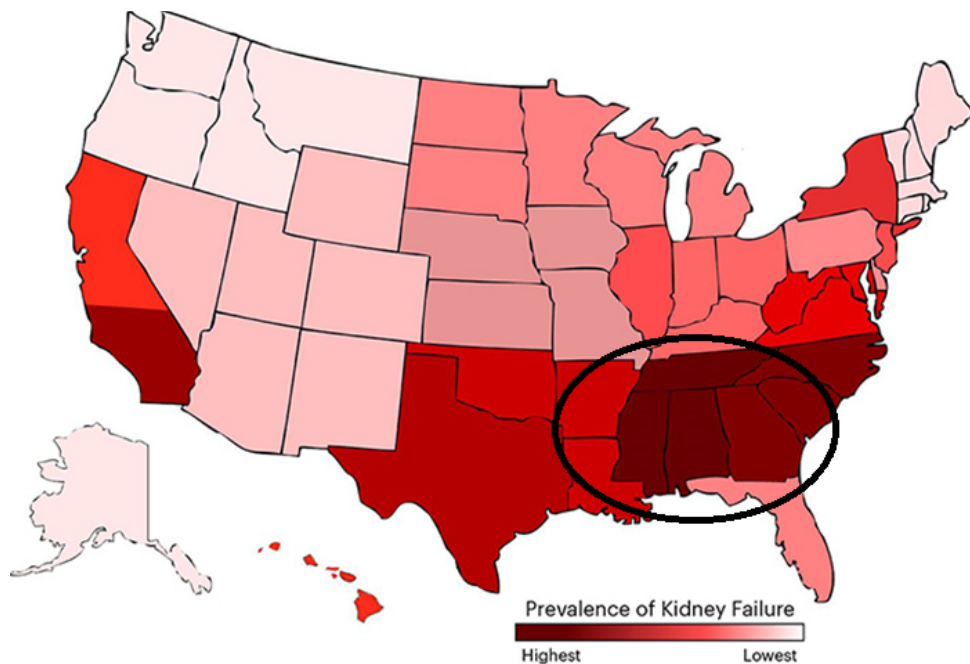
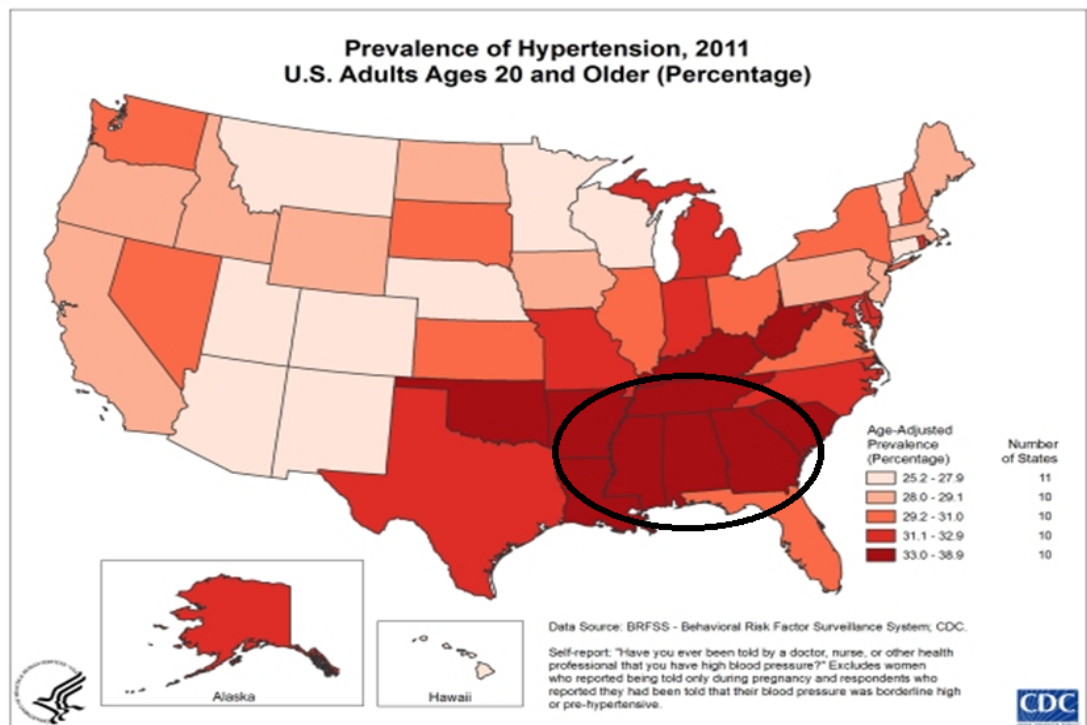


Photo courtesy of Kidney.org

Figure 2. Correlation between LBW, Hypertension, and CKD in Southeastern USA

Researchers have found that there is a health disparity component to CKD and ESRD. Minorities have higher rates of CKD and ESRD compared to whites. Specifically, African Americans, Native Americans, Asian Americans, and Hispanics are respectively 3.9, 2.7, 1.6 and 1.5 times more likely to develop ESRD (5) (Fig 3).

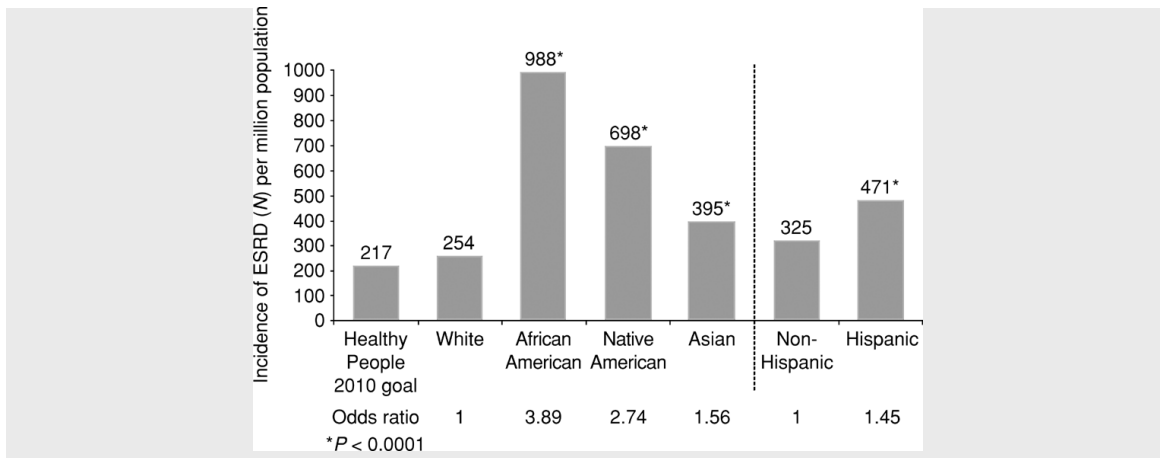


Figure 3. The incidence of reported end-stage renal disease (ESRD) in the United States in 2001, by race and ethnicity adjusted for age and gender ⁴. Data from the United States Renal Data System 2003.

African Americans in the Southeastern United States, and especially Mississippi, are estimated to be five times more likely to develop ESRD associated with hypertension as compared to whites (6).

In 2003, Dr. Alexander's lab at UMMC developed a LBW rat model with increased incidence of adult onset hypertension. For this model, silver clips are placed around the abdominal aorta and uterine arteries in the last third of gestation (Fig. 4). These clips lead to a 35%-45% reduction in uterine perfusion (7) and thus restricted nutrient supply to the developing fetus. It is not known how exactly these LBW rats become hypertensive, but many speculate that low nephron number and the ensuing hyper-filtration of the remaining nephrons and/or altered renal renin-angiotensin-

aldosterone system (RAAS) are contributing factors in this induced uterine growth restriction (IUGR) model (8).

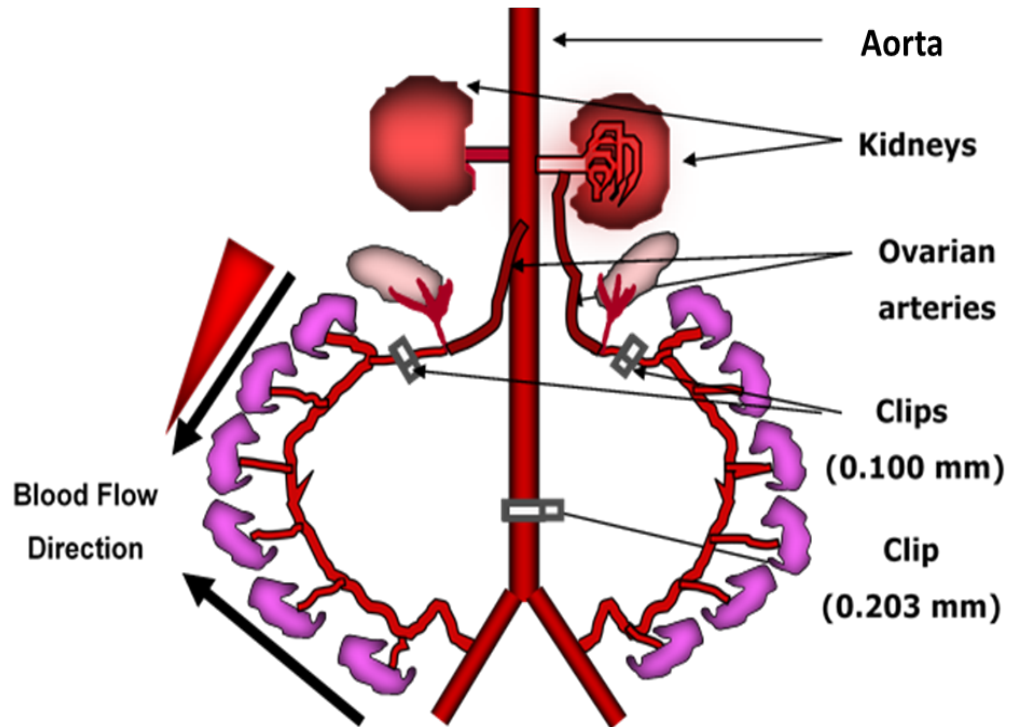


Figure 4. Reduced Uterine Perfusion Pressure surgery. Silver clips are placed on the abdominal aorta and uterine arteries approximately two weeks after fertilization in order to restrict blood flow to the developing fetus. The reduced nutrient availability results in slower fetal development and low birth weight offspring.

Nephrons (functional unit of the kidney) start to form from day 30 of gestation in humans and their development ends at week 36. In essence, humans are born with a full complement of nephrons (9). In rats, the majority of nephrons are established prior to birth, which makes these animals a reasonably good model to study the maternal environment-renal function-hypertension relationship. Prior to placing the clips at the 3rd trimester, approximately 40% of the full nephron complement has been established (10). Restricted nutrient availability and other factors that contribute to low birth weight result

in kidneys that are not completely developed approximately 25-40% fewer number of nephrons (11-13) than normal weight newborns. The deficit in nephron number may lead to glomerular hyperfiltration within the existing nephrons to compensate (14-15). Single nephron hyperfiltration may cause the proximal and/or distal tubule hypertrophy leading to a greater proportion of Na^+ reabsorption, which will increase osmotically driven H_2O reabsorption. The excess H_2O reabsorption can ultimately contribute to increased blood volume and thus cause hypertension to develop. This process is known as the hyperfiltration theory, and many think this leads to a reduction in arteriolar resistance and increased glomerular blood flow.

The RAAS plays a pivotal role in arteriole resistance and the composition, and volume, of the extracellular fluid and is thus one of the candidate mechanisms which might be dysregulated in LBW animals (16). Extracellular fluid sodium concentration is regulated by the RAAS. The process begins by release of renin by the granular cells in the juxtaglomerular apparatus in nephrons of the kidney. Renin is a proteolytic enzyme that cleaves angiotensinogen into angiotensin I. Angiotensinogen is the inactive form of angiotensin I. Angiotensinogen is always present in the plasma of the blood in high concentrations. Angiotensin I passes through the lungs and is converted into angiotensin II by angiotensin-converting enzyme (ACE). ACE is present in the pulmonary capillaries. Angiotensin II is the primary stimulus for secretion of aldosterone from the adrenal cortex. Aldosterone increases sodium reabsorption in the distal and collecting tubules of the nephron (17). Angiotensin II also constricts systemic arterioles which directly increases blood pressure by increasing total peripheral resistance (TPR). The RAAS activity increases salt and water retention and a rise in arterial blood pressure occur (Fig. 5). Despite RAAS being implicated in hypertension of LBW animals and humans, there are conflicting reports indicating it is either up-regulated or down-regulated (8).

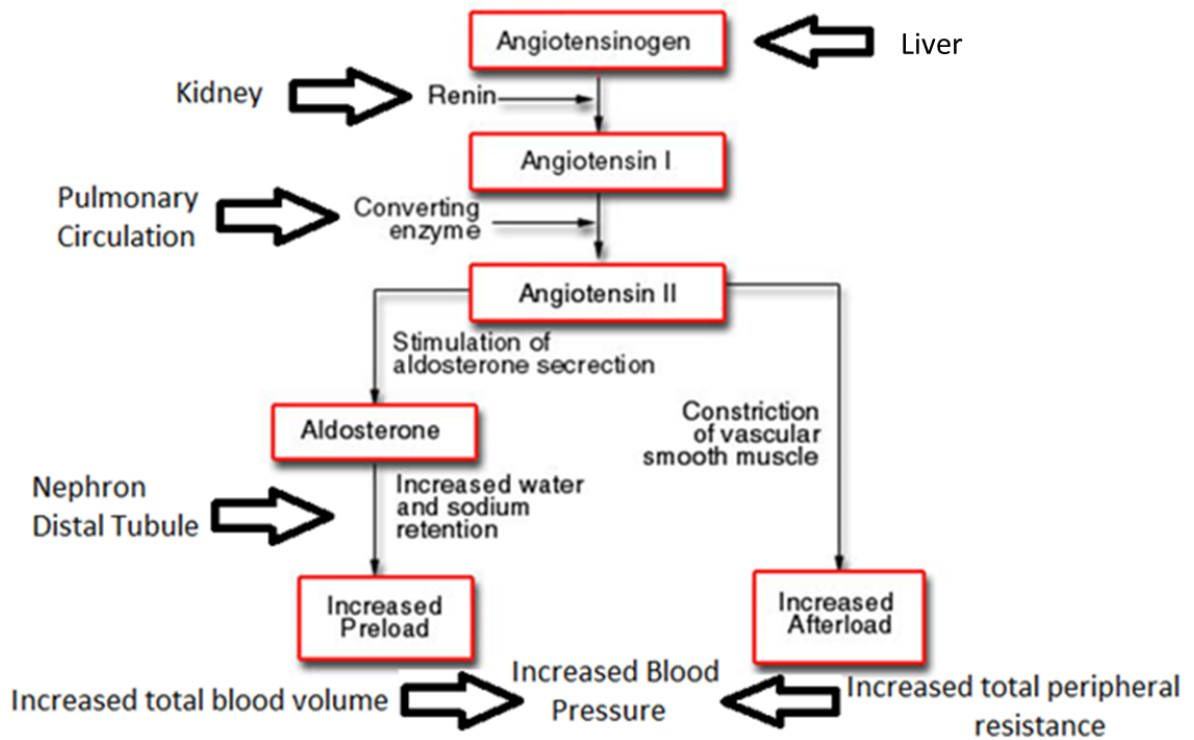


Figure 5. Diagram of the Renin-Angiotensin-Aldosterone System

Once hypertension develops in the IUGR model, they begin to develop CKD. The reduced nephron endowment leads to hyperfiltration in the remaining glomeruli as a compensatory response. Hyperfiltration ultimately leads to fibrosis of the kidneys. Fibrosis of the kidney further damages the kidney and leads to renal disease, further fibrosis, and un-regulation of blood pressure (Fig. 6).

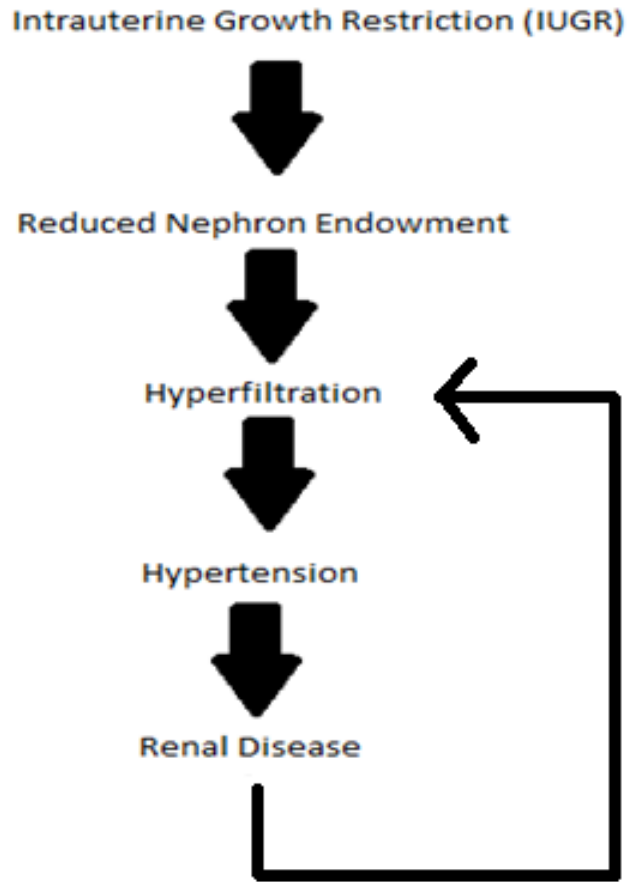


Figure 6. Overview of Renal Disease Development in Low Birth Weight condition. IUGR results in reduced nephron endowment. Having a lower nephron count increases your propensity to hyperfiltrate and develop hypertension. Hypertension contributes to renal disease and renal disease contributes to increased hypertension leading to a positive feedback loop.

My research was focused on determining if anti-hypertensive medication prevents the characteristic decline in renal function that precedes end stage renal disease common to low birth weight animals. Renal function assessed by glomerular filtration rate was evaluated under both basal and acutely stressed conditions. The stressor consisted of transient renal ischemia, a condition from which healthy kidneys can typically recover. We hypothesized that the anti-hypertensive drugs Reserpine and Hydralazine would prevent hypertension and improve renal function in low birth weight rats.

Methods

All experimental procedures proposed in this study were performed in accordance with National Institutes of Health guidelines for use and care of animals with approval of all protocols by the Animal Care and Use Committee at the University of Mississippi Medical Center, as described previously [1]. Briefly, rats were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle with food and water available *ad libitum*. Timed pregnant Sprague Dawley rats were purchased from Harlan Inc (Indianapolis, IN). At day 14 of gestation, rats destined for reduced uterine perfusion were clipped as described below and also in Figure 4. All dams were allowed to deliver at term (21-23 days of gestation) with offspring's birth weight recorded within 12 hours of delivery. In total, there were 32 control offspring and 32 LBW offspring; however, only males are reported so n=16 for control offspring and n=16 for LBW offspring. At this time, the number of pups in the control and reduced uterine perfusion litter were culled to 8 pups per dam to ensure equal nutrient access for all offspring. Animals were weighed weekly until the end of experiments at 12 weeks of age. Pups were weaned at 3 weeks of age. Adult male rats from 8 control pregnant dams and 8 reduced uterine perfusion pregnant dams were randomly assigned into eight groups with n= 4 for each group. Male Control Untreated-Sham (MCUTS); Male Control Treated-Sham (MCTS); Male Control Untreated-Ischemia Reperfusion (MCUTIR); and Male Control Treated-Ischemia Reperfusion (MCTIR) from control dams, and Male Restricted Untreated-Sham (MRUTS); Male Restricted Treated-Sham (MRTS); Male Restricted Untreated-Ischemia Reperfusion (MRUTIR); and Male Restricted Treated-Ischemia Reperfusion (MCRTIR) from dams exposed to reduced uterine perfusion.

Reduced uterine perfusion in the pregnant rat. Using the method previously described, reduced placental perfusion was used to induce low birth weight offspring [1].
Briefly, all

rats undergoing surgical procedures were anesthetized with 2% isoflurane (W.A. Butler Co., Memphis, TN) delivered by an anesthesia apparatus. At day 14 of gestation, a silver clip (0.203-mm ID) was placed around the abdominal aorta above the iliac bifurcation. To avoid compensation of blood flow from the ovarian arteries, silver clips (0.100mm ID) were placed on both branches of the ovarian arteries. Pregnant rats not exposed to surgical procedure were used as the control pregnant counterpart group and designated as the sham operated group.

Drug administration: Two drugs with effects on cardiovascular function were used to lower blood pressure. Hydralazine: was administered via drinking water at a dose of 80mg/L initiated at 6 weeks of age until the end experiment at 12 weeks of age. Reserpine: was administered via drinking water at a dose of 5mg/L initiated at 6 weeks of age until the end of experiments at 12 weeks of age. Administration of this medication constituted the “treated” rats.

Ischemia/Reperfusion (I/R) renal Injury. Adult male rats at age of 12 weeks were exposed to bilateral mild I/R as previously described [1]. Briefly, with animals under isoflurane anesthesia, bilateral mild I/R were induced by occluding both renal pedicles with micro vascular clamps for 15 minutes (mild ischemia). Completeness of ischemia was verified by blanching of the kidneys, which is an indication of the stoppage of blood flow. The blood flow to the kidneys was reestablished by removal of both clamps (reperfusion) with visual verification of blood return by changing in the kidney’s color to a homogeneous dark pink. The sham animals were subjected to the same procedure except the renal pedicles were not clamped. After the I/R procedure, animals were instrumented for renal hemodynamic measurements as described below. The abdominal cavity was closed in two layers (muscles and skin) and the animals were placed in separate cages for recovery. The body temperature of the animals was monitored and maintained stable during the whole procedure using a rectal thermometer in sync with a heating pad.

Measurement of systemic and renal hemodynamics. As previously described [1], rats under isoflurane anesthesia were surgically instrumented with flexible catheters (PE 50 tubing) in the right jugular vein for infusion and in the right carotid artery for measurement of arterial pressure and collection of blood. All catheters were tunneled to the nape of the neck and exteriorized. Renal function and arterial pressure measurements were performed in the conscious state after a 2hs recovery phase from the ischemic event. Mean arterial pressure (MAP) was monitored in conscious, chronically instrumented rats via connection of the arterial catheter to a pressure transducer and a data set (PowerLab 16/30) using the software Lab Chart Pro V7 both from ADInstruments, Pty. Ltd. The data acquisition set was connected to a PC for continuous recording.

Glomerular filtration rate (GFR) was calculated from serial measurement of the concentration of fluorescein isothiocyanate (FITC)-inulin (from Sigma Aldrich, San Louis MO Cat# F3272) in plasma, collected every 15 minutes at 4 time points following 60 minutes of continuous infusion to allow FITC-inulin to reach a steady state plasma concentration. Plasma (FITC) -inulin was measured using a mini-plate fluorescence spectrophotometer reader (Fluorospectrometer Thermo Fisher). Since FITC-inulin is freely filtered at the glomerular capillaries but is not reabsorbed by the nephron tubules, the rate of FITC-inulin filtration is equal to its rate of excretion in the urine, which is the product of urine formation rate (UFR) and the urine concentration of FITC-inulin (Equation 1).

$$\text{GFR} \times [\text{FITC-inulin}]_{\text{plasma}} = \text{UFR} \times [\text{FITC-inulin}]_{\text{urine}}, \quad (1)$$

Under conditions where the plasma concentration of FITC-inulin has reached a steady-state during constant infusion of a stock solution into the circulation (CIR), then the rate of infusion is equal to the rate of excretion (Equation 2).

$$\text{UFR} \times [\text{FITC-inulin}]_{\text{urine}} = \text{CIR} \times [\text{FITC-inulin}]_{\text{stock}} \quad (2)$$

FITC-inulin was prepared as a stock solution at a concentration of 6 mg/mL and was infused into the circulation at 0.05 mL/min. Hence, GFR was calculated from Equation 3.

$$GFR = \frac{0.3 \text{ mg/min}}{[FITC - inulin]_{\text{plasma}}} \quad (3)$$

The value of GFR obtained with this calculation was adjusted by total kidney weight for each animal.

Statistics. Microsoft Excel was used for all statistical analysis. A value of $P < 0.05$ was considered statistically significant.

Results

Fetal blood flow was restricted during the final 7-8 days of *in utero* development by surgically implanting three clips onto the abdominal aorta and ovarian arteries of isoflurane-anesthetized rats (see Fig. 4). In total, eight pregnant females were uterine restricted, yielding thirty-two mixed gender offspring, all of which were viable. For this study, a total of thirty-two male offspring (n=16 controls and 16 uterine restricted) were used. The uterine restricted rats had normal gestations, but weighted significantly less than the controls (Fig. 7).

The rats were divided into four groups and weighed weekly in anticipation of treating half of the control and uterine restricted rats with anti-hypertensive drugs beginning at week 6 (Fig. 8). By two-way repeated measures ANOVA, there was a significant main effect of time, but no significant treatment effect on body mass gained per week, indicating that the uterine restricted rats seemed to develop normally after birth. There was a borderline time x treatment interaction ($p=0.055$), which could be attributed to small differences between the groups in body mass gained before vs. during administration of the anti-hypertensive drugs. Most notably, from weeks 1-4 before administration of the drugs, both groups of uterine restricted rats gained mass at nearly the same rate as the controls, but from weeks 9-12 the uterine-restricted rats given anti-hypertensive medication gained slightly more weight (about 0.2 g/week) than the treated controls while the uterine-restricted rats not given the medication gained slightly less weight (about 2 g/week) than the corresponding untreated controls. Thus, the anti-hypertensive medication Reserpine and Hydralazine appeared to have a small positive effect on weekly weight gain in the uterine restricted animals.

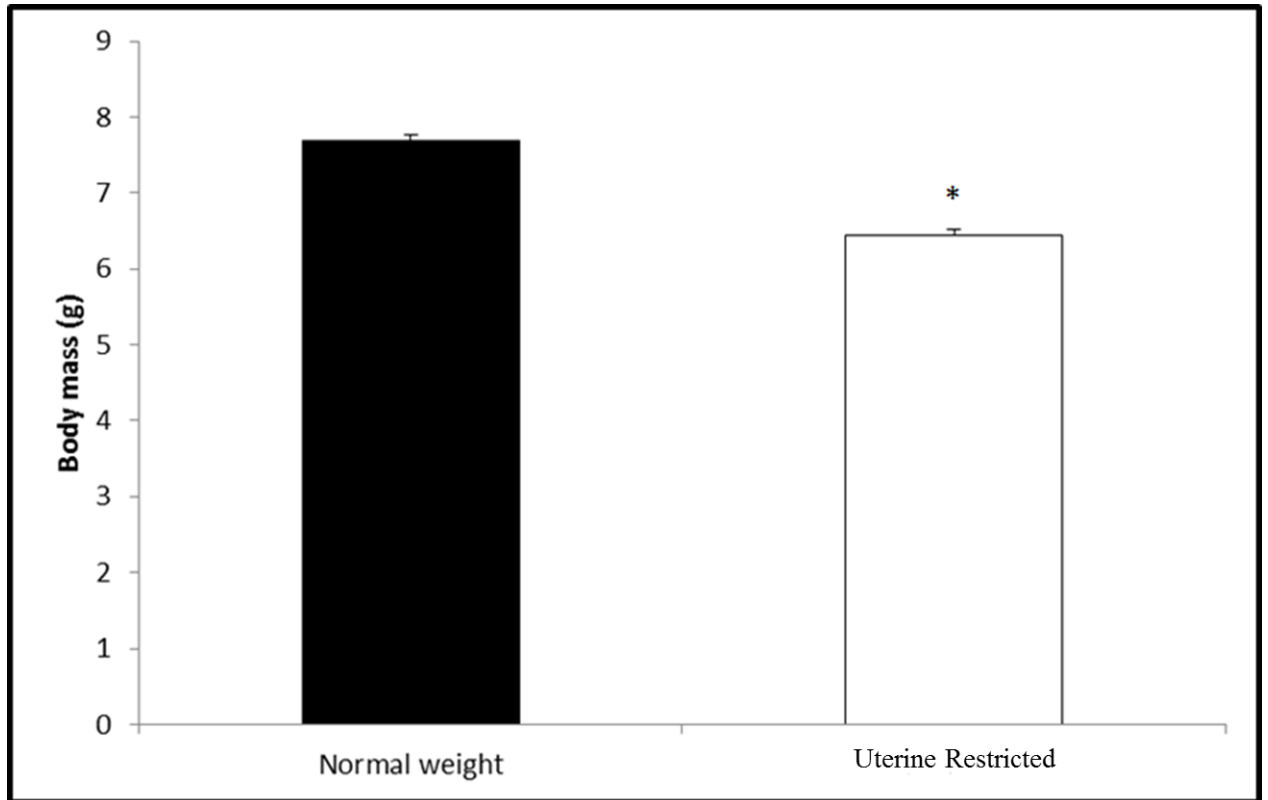


Figure 7. Body mass of newborn rats from control and uterine blood flow-restricted mothers. Statistical analysis was performed using a two-tailed t-test on two groups. Data are mean \pm S.E.M. of 16 experiments; * $p < 0.001$.

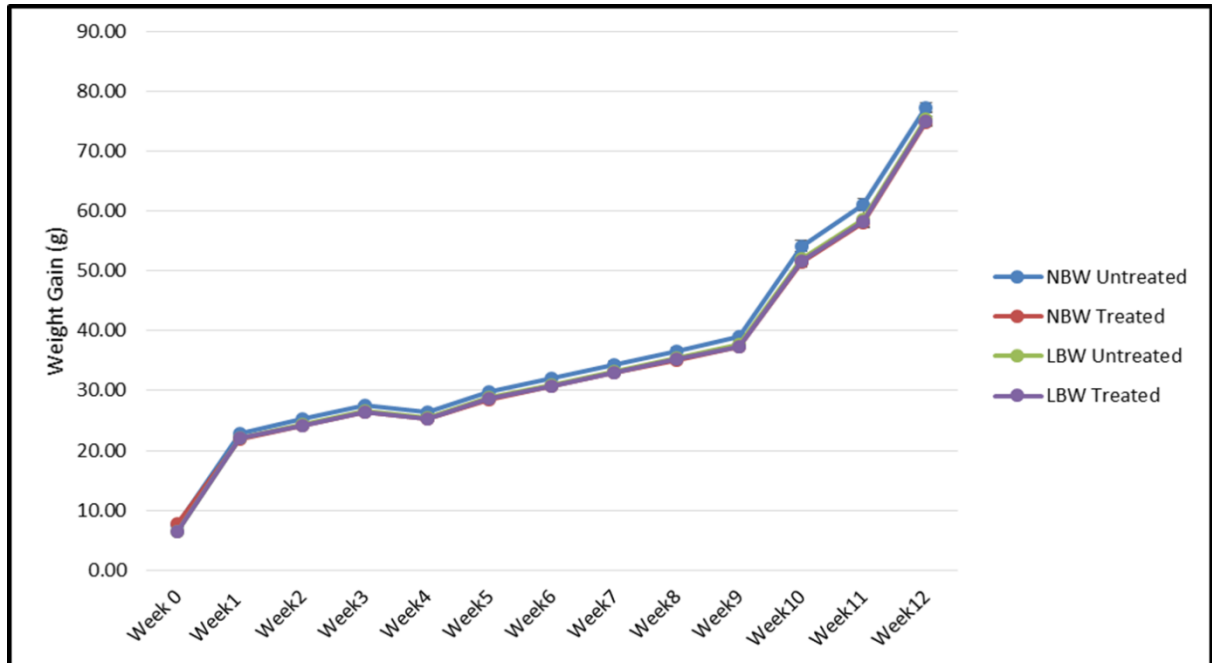


Figure 8. : Weekly body mass gained of control (NBW) and uterine restricted (LBW) rats either treated or not treated with anti-hypertensive medication. The rats were weighed weekly to assess post-natal development. The anti-hypertensive drugs Reserpine and Hydralazine were added to the drinking water (5 mg/L and 80 mg/L, respectively) of the treated animals beginning at week 6. Analysis was performed using a two way repeated measures ANOVA (time and treatment as the factors); n=8 per group. There was a significant main effect of time ($p<0.001$) but not treatment ($p=0.248$), but there was a borderline significant time x treatment interaction ($p=0.055$).

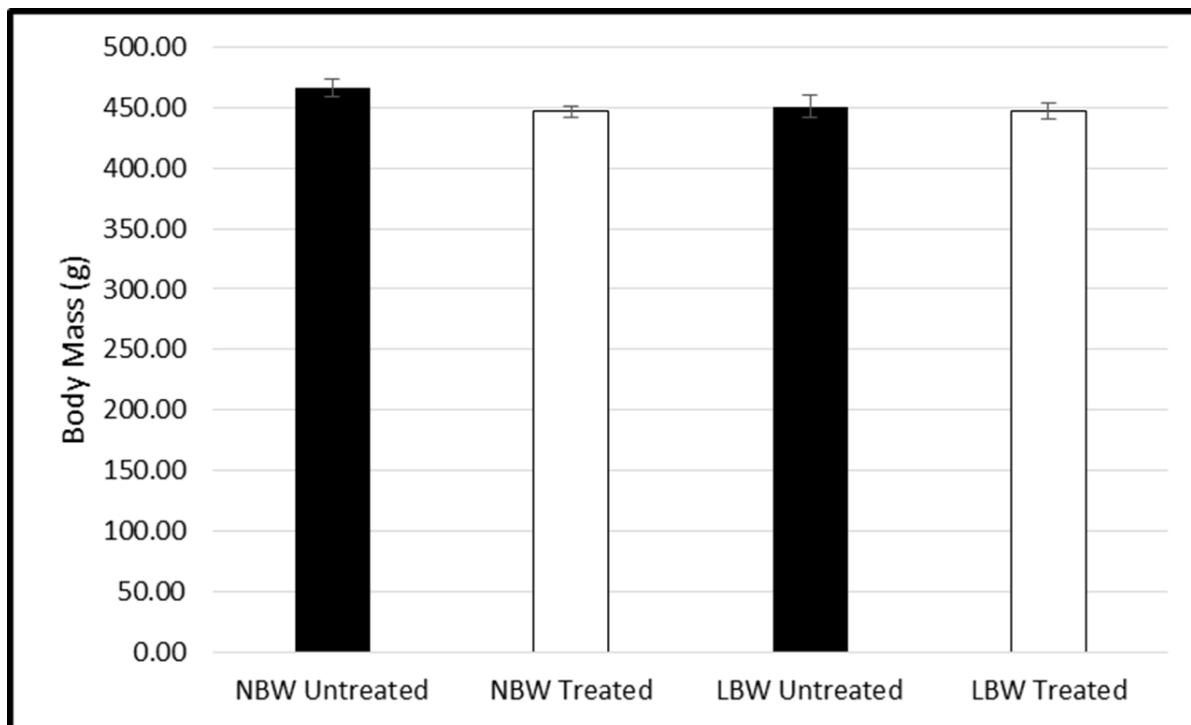


Figure 9. Final body mass of control (NBW) and uterine-restricted (LBW) rats either treated or not treated with anti-hypertensive medication for six weeks. The data were analyzed by 1 way ANOVA. There were no significant differences between the groups. Data are mean \pm S.E.M. of eight experiments.

Final week 12 body masses were analyzed by two way ANOVA, with uterine clip (birth weight) and anti-hypertensive medication as the main factors (Fig. 9). There was no significant main effect of birth weight on body mass ($p=0.352$). There was also no significant effect of the medication on body mass ($p=0.140$). There was also no birth weight x drug interaction ($p=0.291$), so the effect of the medication did not depend on whether the animals had been uterine-restricted. One-way ANOVA confirmed that the final body masses did not differ significantly between the groups ($p=0.248$)

After the final body masses were taken, the animals were surgically implanted with a catheter into the right carotid artery for measurement of mean arterial pressure following 2h recovery from the anesthesia (Fig. 10). From two-way ANOVA, there were

highly significant main effects of uterine restriction ($p<0.001$) and anti-hypertensive medication ($p<0.001$), as well as a highly significant ($p<0.001$) uterine clip x medication interaction.

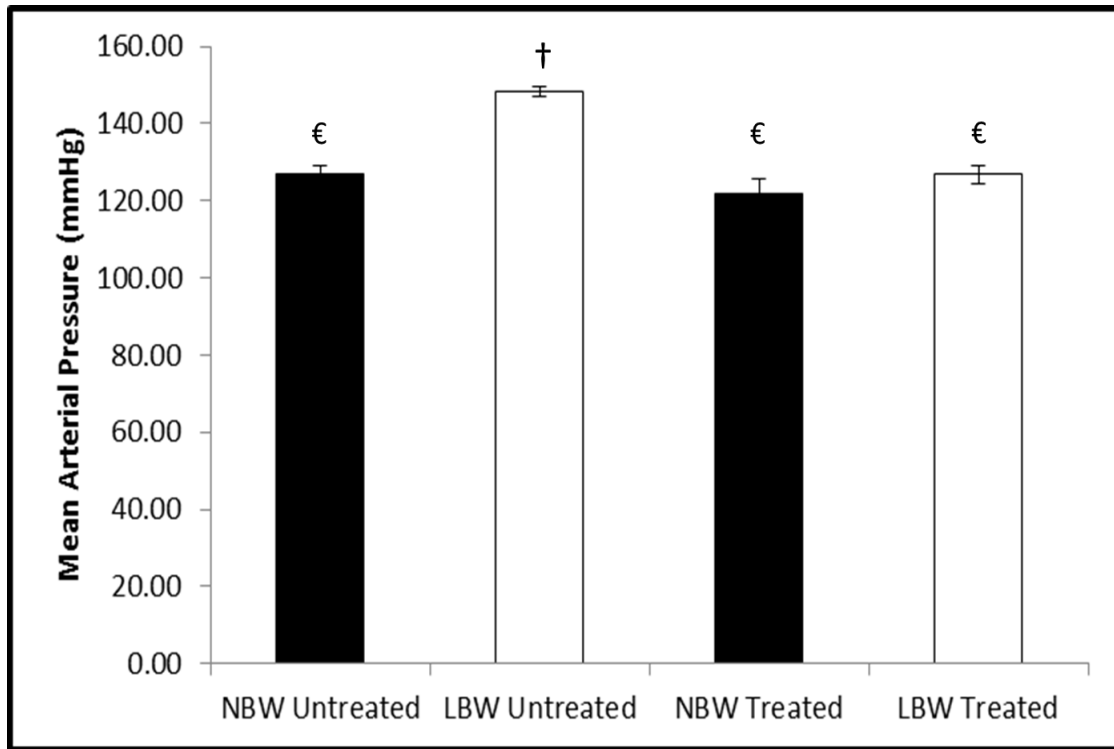


Figure 10. Mean arterial pressure of control (NBW) and uterine-restricted (LBW) conscious rats treated for six weeks with anti-hypertensive medication. The data were analyzed by two-way ANOVA, followed by one-way ANOVA and Tukey's post-hoc test. Means sharing a common symbol did not significantly differ. Data are mean \pm S.E.M of eight experiments.

To determine which groups differed, a one way ANOVA was performed using the 4 groups ($n=8$ per group). This also yielded a highly significant p value ($p<0.001$), and Tukey's post-hoc test indicated that the uterine restricted rats not treated with hypertensive medication had significantly higher MAP than the other groups, confirming that low birth weight animals develop hypertension as adults.

Next, the animals were divided into two additional groups to assess kidney function under both unstressed and stressed conditions. The rationale for this was that twelve week old uterine restricted rats may have subtly altered or weakened kidney function that may be difficult to detect under normal conditions, as they are still relatively young. However, subtle defects might be amplified, and thus more likely to identify, after a physiological stressor. For this study, the kidneys were subjected to a 15 min transient ischemia followed by 2 h recovery. Glomerular filtration rate (Fig. 12) and combined kidney mass (Fig. 11) were then measured.

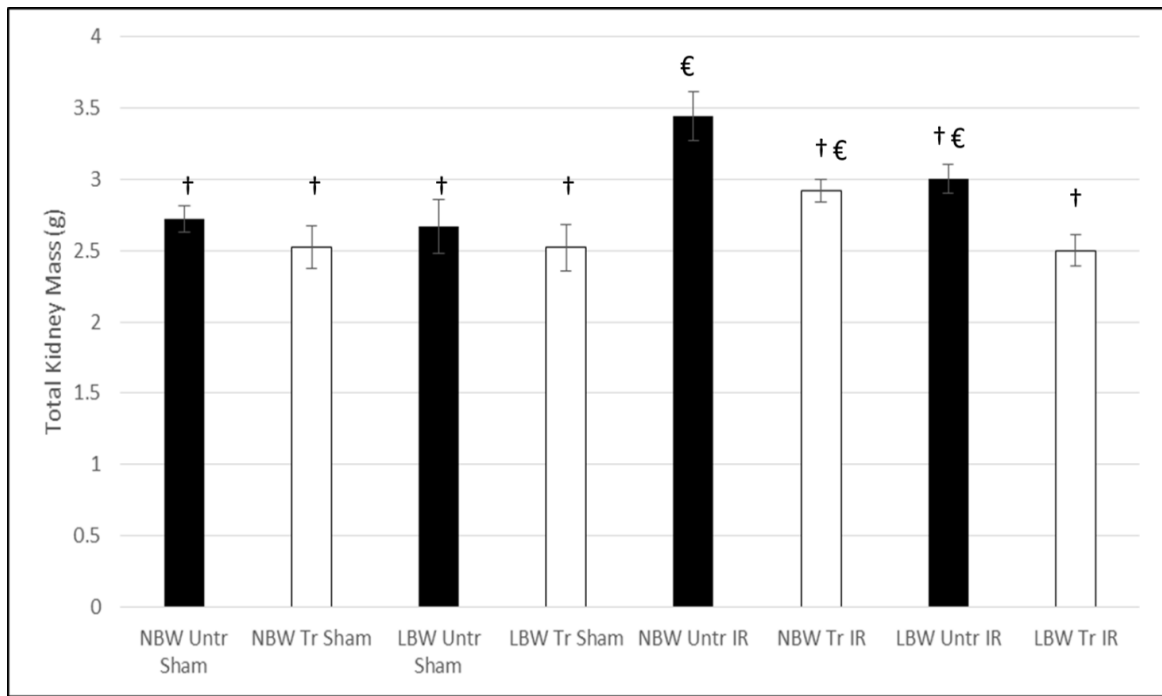


Figure 11. Kidney mass of control (NBW) and uterine-restricted (LBW) rats subjected to anti-hypertensive medication for six weeks and/or acute ischemia-reperfusion (IR). The data were analyzed by two-way ANOVA, followed by one-way ANOVA and Tukey's post-hoc test. There was a significant effect of the treatments ($p=0.001$). Means sharing common symbols do not significantly differ. Data are mean \pm S.E.M. of 4 experiments.

A two way ANOVA (n=8 per group) was performed using uterine restriction and acute stressor (IR) as the two factors. There was a significant main effect of IR ($p=0.004$), with this stressor increasing kidney mass. There was a nearly significant main effect of birth weight ($p=0.061$), where low birth weight animals tended to have lower kidney mass. There was no significant IR x birth weight interaction ($p=0.090$), but this was close, as the effect of ischemia reperfusion on kidney mass tended to be greater in the NBW rats than in the LBW rats. When analyzed in this way, the mean kidney masses were: NBW sham 2.62 ± 0.09 g; NBW IR 3.18 ± 0.13 g; LBW sham 2.59 ± 0.12 g; and LBW IR 2.75 ± 0.12 g.

When the 2 way ANOVA was run using uterine restriction and anti-hypertensive medication as main effects: there were significant effects of both the drugs ($p=0.002$) and uterine restriction ($p=0.010$), but no significant drug x birth weight interaction ($p=0.414$). From this analysis, the mean kidney masses were: NBW no drug: 3.08 ± 0.16 g; NBW plus drugs: 2.72 ± 0.11 g; LBW no drug: 2.84 ± 0.12 g; LBW plus drugs: 2.51 ± 0.09 g. So BP drug treatment significantly lowered kidney mass, and this effect did not depend on birth weight.

A one-way ANOVA was used to determine which means differed significantly (Fig. 11). None of the unstressed groups exhibited differences in kidney mass. It was somewhat surprising that the uterine-restricted, sham operated rats did not have significantly lower kidney mass, as these animals have been shown to have a reduced number of nephrons (insert reference). There was a tendency for the unstressed animals treated with anti-hypertensive medication to have lower kidney mass, but with only four animals per group, the differences were not sufficiently robust to be significant.

Subjecting the kidneys to acute IR significantly increased kidney mass in both groups not treated with Reserpine and Hydralazine. Control untreated rats showed the greatest response, while the uterine restricted untreated rats showed a somewhat attenuated response that did not significantly differ from the stressed controls. Chronic

treatment with the anti-hypertensive medications attenuated the increased mass for both control and uterine-restricted groups but neither effect was significantly different from the corresponding untreated groups. An analysis using kidney mass normalized to body mass was also performed, and this yielded the same results, so the outcome did not matter if the absolute kidney mass or kidney mass normalized to body mass was used.

Glomerular filtration rate is an important measure of kidney function, as it reflects the rate at which waste products are excreted from the body via the urine. This rate depends on a few key variables, including mean arterial pressure, plasma colloid osmotic pressure, glomerular capillary integrity, and the total number of functional nephrons. Since low birth weight animals have been shown to have 25-40% fewer nephrons, total GFR was predicted to be lower in uterine restricted, low birth weight rats, but this was not the case (2.57 ± 0.18 mL/min vs. 2.54 ± 0.28 mL/min for control vs. uterine-restricted untreated, unstressed rats; $n=4$). The higher MAP, which tends to increase GFR, in the uterine-restricted rats may partly explain the lack of difference. Mass-normalized GFR can provide additional insight into nephron function under conditions where differences in nephron number might occur. For this reason, GFR per mass kidney was analyzed (Fig. 12).

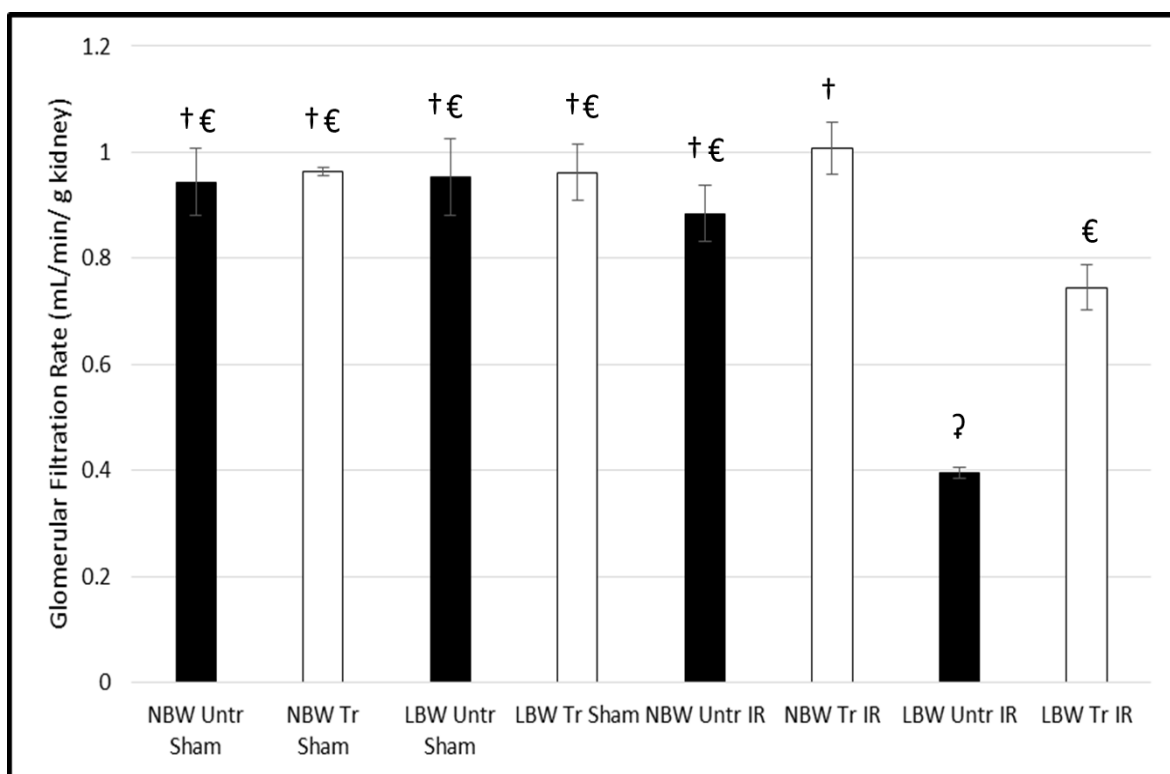


Figure 12. Glomerular filtration rate of control (NBW) and uterine-restricted (LBW) rats subjected to anti-hypertensive medication for six weeks and/or acute ischemia-reperfusion (IR). The data were analyzed by two-way ANOVA, followed by one-way ANOVA and Tukey's post-hoc test. Means sharing common symbols do not significantly differ. Data are mean \pm S.E.M. of 4 experiments.

A 2-way ANOVA using uterine restriction and IR as main factors yielded a significant main effect of IR ($p < 0.001$) and of uterine restriction ($p < 0.001$). There was also a significant interaction ($p < 0.001$), so the effect of ischemia-reperfusion depended on whether the animals were low or normal birth weight. This is readily apparent, as GFR/g was substantially impaired in the uterine restricted animals but not the controls: NBW sham: 0.954 ± 0.030 mL/min/g kidney, NBW IR: 0.946 ± 0.041 mL/min/g kidney,

LBW sham: 0.958 ± 0.041 mL/min/g kidney, LBW IR: 0.571 ± 0.069 mL/min/g kidney (n=4).

If the two factors used are uterine restriction and anti-hypertensive drugs, then there was a significant main effect of drugs ($p=0.002$) and of uterine restriction ($p<0.001$), and a significant drug x uterine restriction interaction ($p=0.013$), so the effect of the BP drugs on GFR/g kidney depended on whether the animals were low or normal birth weight. Reserpine and Hydralazine had more pronounced effects on enhancing GFR/g in the uterine-restricted than control animals: NBW no BP drugs: 0.914 ± 0.040 mL/min/g kidney (n=8), NBW plus BP drugs: 0.986 ± 0.024 mL/min/g kidney (n=8), LBW no BP drugs: 0.675 ± 0.111 mL/min/g kidney (n=8), LBW plus BP drugs: 0.853 ± 0.052 mL/min/g kidney (n=8). It is apparent that addition of the BP drugs increased GFR/g kidney more in the LBW rats than in the NBW rats, and thus the significant interaction.

To determine which groups significantly differed, a one-way ANOVA with Tukey's post hoc test was performed. Surprisingly, GFR/g in the uterine-restricted, unstressed, untreated animals was not significantly higher, as predicted from both the higher MAP and presumably lower nephron number in these rats. Thus, there was no evidence of significant hyperfiltration occurring, and so this seemingly cannot explain the hypertension which developed. It was also surprising that Reserpine and Hydralazine significantly lowered MAP in the uterine restricted rats without affecting GFR/g, given that MAP is a primary driver of GFR. Stressing the kidneys with transient ischemia, however, did result in evidence for subtle changes in kidney physiology, as IR resulted in a significant fall in GFR/g in the uterine restricted but not the control animals. Prior treatment of the animals for six weeks with anti-hypertensive medication significantly attenuated the stress-induced decline in GFR, much more so in the uterine-restricted animals than the controls. Thus, uterine-restriction and the resulting low birth weight had long-term adverse effects on kidney function that seemed to be further exacerbated by the developing hypertension. The kidney dysfunction could not readily be detected under unstressed conditions by measurement of GFR, but was readily apparent after transient

IR. This adverse effect may contribute to the hypertension and ensuing pathologies that develop in these animals.

Discussion

Rats born in the IUGR model had a statistically significant lower birth weight compared to rats born in the normal environment (Fig. 7). The low birth weight rats gained weight normally and so weighed the same as control rats at twelve weeks of age (Fig 8 and 9). This observation is seen in humans as well. Although not measured, the nephron number in the LBW rats should be lower compared to the NBW rats.

The weight gained in any given week may significantly depend on how the animals were treated. So, despite the fact that there was no significant effect of the treatments on weight gain (which does not take into account weekly differences), there might be differences between the groups for some weeks but not others. This became apparent when comparing low birth weight to normal birth weight untreated rats- the difference in weekly weight gain increased as the animals get older. For the LBW rats, for weeks 1-4 their average weekly weight gain was about 0.8-0.9 grams less than the NBW rats, but for weeks 9-12 the average weekly weight gain for LBW rats was a bit more- about 1.5 to 2.2 grams less than the NBW rats. Contrast this to the NBW and LBW drug treated rats- here during weeks 1-4 the LBW rats gained virtually identical weight to the NBW rats, but for weeks 9-12, the drug treated LBW rats gained slightly more weight (just about 0.2 g per week) than the treated NBW rats. So, the anti-hypertensive medication appeared to positively influence weight gain in the LBW rats once the rats got older (without the drug treatment weeks 9-12 weight gain was 1.5-2.2 g less than NBW but with drug weight gain was 0.2 g per week more than drug treated NBW). This makes sense given how the experiment was run: the animals were not given the BP drugs until week 6, so if there was to be any effect of the drug, it would not be until after week 6, and that's what the nearly significant interaction is indicating. The effect of the anti-

hypertensive medication was too small to yield any significant main effect between the treatment groups (not considering the

differences each week), and this can also be partly explained by the fact that the animals were not treated with the drugs through weeks 1-5. Overall, Reserpine and Hydralazine treatment for 6 weeks appeared to have a small, although not significant, positive effect on increasing weight gain in low birth weight rats.

Mean Arterial Pressure (MAP) is an important regulated physiological variable that partly determines the rate of blood flow into each systemic tissue. It is the average pressure driving blood forward into the tissues throughout the cardiac cycle and is defined as the addition of two thirds of diastolic pressure and one third of systolic pressure. Diastolic pressure is the pressure at the end of ventricular diastole and systolic pressure is the peak pressure that occurs during ventricular systole. Short term changes in MAP are typically due to changes in sympathetic tone to the arterioles and/or heart, or to changes in the availability of metabolically-produced paracrine factors that influence the arterioles. Long term regulation of MAP depends primarily on hormonal control of Na^+ reabsorption and distal tubule water permeability within the nephrons of the kidneys. Variable Na^+ and water reabsorption in the kidneys is an important factor that affects total blood volume, which is one determinant of MAP.

The juxtaglomerular apparatus in nephrons of the kidneys allows for regulation of long term MAP. The juxtaglomerular apparatus is a specialized combination of thick ascending tubular cells (the macula densa cells) and vascular cells (the granular cells) that connects the afferent arterioles, efferent arterioles, glomerulus, and ascending limb of the Loop of Henle. The afferent arteriole granular cells of the juxtaglomerular apparatus secrete renin into the blood in response to a decrease in the filtrate concentration of NaCl , the extra-cellular fluid volume, or arterial blood pressure. When renin is secreted into the blood stream it catalyzes proteolysis of the peptide prohormone angiotensinogen into angiotensin I, which is then further digested by the lung-localized angiotensin converting enzyme into angiotensin II (AII) (Fig. 5). Angiotensin II induces aldosterone synthesis and secretion by the adrenal cortex endocrine cells, which promotes Na^+ reabsorption in the distal tubule of the nephron. Increased Na^+ reabsorption enhances the osmotic gradient across the distal tubule, thereby stimulating greater water reabsorption.

In low birth weight animals, there has been speculation that the juxtaglomerular apparatus develops abnormally, resulting in dysfunctional regulation of renin release from the granular cells. Macula densa cells of the ascending Loop of Henle stimulate renin exocytosis in the afferent arteriole by releasing at least two important paracrine factors: prostaglandin E2 (PGE2) and nitric oxide (NO). Both factors increase granular cell cAMP levels (through activation of prostaglandin E4 receptors and guanylyl cyclase, respectively), which induce exocytosis of renin through activation of protein kinase A. Increased release of these paracrine factors occurs when the tubular filtrate concentration of NaCl (Cl^- is thought to be more important) to which the macula densa cells are exposed is abnormally low. Conversely, the cells release adenosine when the filtrate concentration of NaCl is abnormally high, and this appears to inhibit renin release through A1 adenosine receptors (18, 19). The macula densa cells in low birth weight rats have been suggested to have altered responses to tubular NaCl, causing them to release abnormally high amounts of renin under normal concentrations of NaCl. However, the results from multiple studies have shown conflicting results on whether the renin-angiotensin system is overly active in low birth weight animals (8).

We cannot fully make the claim that LBW rats have lower kidney mass than NBW rats from the data presented in figure 11. Low birth weight animals tended to have lower kidney mass; however, the results were not significant. Kidney mass may directly correlate with the number of nephrons, so the uterine-restricted rats in this study may not have a significantly lower nephron number compared to the normal birth weight rats. The GFR measurements tended to agree with this, as total GFR in LBW rats was not significantly different from the control rats, as might be expected with a lower number of nephrons. However, the increase in MAP observed in the LBW rats promotes increased GFR since glomerular pressure is a major driving force for glomerular capillary filtration, so a possible loss in nephron number could be offset by hyperfiltration at the remaining nephrons. Hyperfiltration was assessed by calculating mass-specific GFR, which could be taken as an index of single-nephron GFR assuming that kidney mass directly correlates

with nephron number. Mass-specific GFR was not increased in the LBW rats, suggesting that hyperfiltration was not occurring.

Glomerular filtration is created via four physical factors- glomerular capillary blood pressure, plasma-colloid osmotic pressure, Bowman's capsule hydrostatic pressure, and the capillary filtration coefficient (a measure of the resistance of the capillary/Bowman's capsule membrane to the movement of water and solutes across the membrane). Glomerular capillary blood pressure is the fluid pressure exerted by the blood within the glomerular capillaries. It is a result of cardiac output and resistance to blood flow of the afferent and efferent arterioles. Glomerular capillary blood pressure favors filtration. Plasma- colloid osmotic pressure is caused by the unequal distribution of plasma proteins across the glomerular membrane. Plasma proteins cannot be filtered so they remain in the glomerular capillaries and not in Bowman's capsule. Because of this, the concentration of H₂O is higher in Bowman's capsule than in the glomerular capillaries and H₂O moves down its concentration gradient through osmosis into the glomerulus. This opposes glomerular filtration. Bowman's capsule hydrostatic pressure is the pressure exerted by the fluid in this initial part of the tubule. This pressure pushes fluid out of Bowman's capsule and opposes the filtration of fluid from the glomerulus into Bowman's capsule. The result of all three of these forces determines the net filtration pressure, but the actual rate of filtration also depends on how easily the water and solutes can move across the capillary/Bowman's capsule membrane, which can change physiologically due to a unknown factors affecting the contractile state of mesangial cells which form the Bowman's capsule membrane.

Plasma-colloid osmotic pressure and Bowman's capsule hydrostatic pressure cannot be controllably altered; however, glomerular capillary blood pressure can be controlled. As the glomerular capillary blood pressure increases, the net filtration increases and so does GFR. Intrinsic regulatory mechanisms, initiated by the kidneys themselves, prevent inadvertent changes in GFR. When there is an increase in GFR due to an increase in arterial pressure, GFR can be reduced to normal by constriction of the afferent arteriole. This causes a decrease in the flow of blood into the glomerulus and a

lowering of GFR to normal levels. When GFR falls as a result of a decline in arterial pressure, e.g. renal ischemia, glomerular pressure can be increased to normal by vasodilation of the afferent arteriole. This allows for more blood and increases GFR to normal. Despite this autoregulation of glomerular capillary pressure, it usually is not completely effective, so that a higher MAP tends to result in a smaller increase in glomerular pressure and thus an increase in GFR. Since the data indicated no increase in mass-specific GFR at the higher MAP, this could indicate the LBW rats have a lower glomerular filtration coefficient which offsets the MAP. It is worth speculating that uterine restriction may partly exert its effect by causing conditions that promote hypersensitivity of mesangial cells to factors which induce their contraction, thus chronically lowering the filtration coefficient. This could impair NaCl filtration, causing overall NaCl retention and thus promoting excess water reabsorption.

When considering the GFR adjusted per kidney weight (Fig. 12), the LBW rats subjected to IR were much more susceptible to impaired GFR than NBW rats, and the prolonged treatment to reduce MAP partially restored GFR, but it was still significantly lower than that in the NBW treated rats subjected to IR. The LBW untreated I/R group simply cannot recover from the mild insult to the kidney. These data further support a possible mesangial cell hypothesis: ischemia-reperfusion induces the release of unknown factors to which the mesangial cells in the LBW rats are hypersensitive, causing further excessive contraction and reduction in the filtration coefficient, and thus the pronounced fall in GFR.

Based on this study, I would advocate using LBW as a biomarker for elevated risk of hypertension and kidney disease. Persons born LBW should know their blood pressure and get this checked at least once a year. Hypertension is known as the “silent killer”. If someone who was a LBW infant did not know they were hypertensive for a long period of time, they could very easily develop kidney disease from our results. This kidney disease would aid in the further development of hypertension and a positive feedback loop would occur. I would suggest physicians begin to ask their patients if they

were born LBW. If so, they should consider placing them on antihypertensive drugs to improve kidney function.

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